RED 660 Protein Assay

A Ready-To-Use Colorimetric Protein Assay

INTRODUCTION

RED 660 Protein Assay is a single reagent colorimetric assay that outperforms commercial colorimetric assays, including Bradford and improved Coomassie/ Bradford assays. RED 660 Protein Assay offers greater linearity, greater color stability, and greater compatibility with detergents, reducing agents and other interfering agents compared to the Coomassie assays. The single, ready-to-use reagent allows for rapid analysis of total protein concentration and generates highly reproducible results.

This assay is suitable for the simple and rapid estimation of protein concentration and detects proteins in the range of 50-2000µg/ml. This assay is based on a single proprietary dye-metal complex reagent. The binding of protein to the dye-metal complex under acidic conditions results in a change of color from reddish-brown to green and this change in color density is proportional to protein concentration. The color change is a result of deprotonation of the dye-metal complex at low pH, which is facilitated by interactions with positively charged amino acid groups. Protein estimation can be performed using as little as 0.5µg protein. The protein-dye complexes reach a stable end point in 5 minutes, remaining stable for several days.

The RED 660 Protein Assay has sufficient reagents for 500 standard test tube assays or 2,500 standard microwell assays.

ITEM(S) SUPPLIED **Cat# 786-676**

Description	Size
RED 660 Protein Assay Reagent	2 x 250ml
Bovine Serum Albumin (BSA) Standard (2mg/ml)	5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Store RED 660 Protein Assay Reagent at room temperature and Bovine Serum Albumin (BSA) Standard at 4°C, upon arrival. When stored and used as recommended, the reagent is stable for one year.

MATERIAL NEEDED BUT NOT SUPPLIED

- Disposable 1ml polystyrene cuvettes (Cat. # 786-009), 2ml assay tubes (Cat. # 786-008)
- Microplate
- Optional: Neutralizer™ (Cat. # 786-673) for samples with >0.01% SDS or in Laemmli buffer

PROTOCOLS

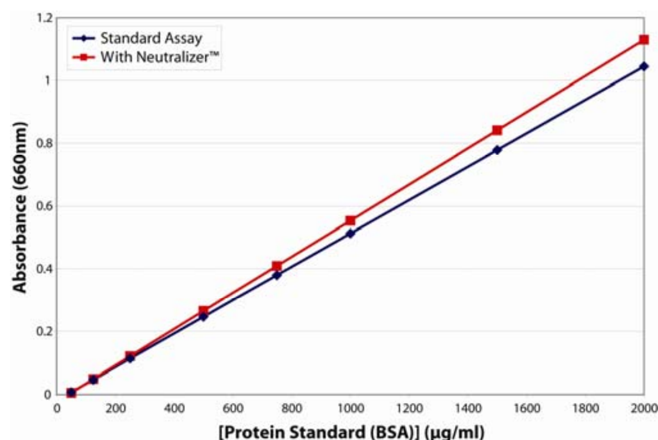
1. Preparation of Protein Standards
2. Sample Preparation
3. Microplate or Microwell (200µl) Assays:
4. Test Tube (1ml) Assays:

1. PREPARATION OF PROTEIN STANDARDS

For minimizing interference, it is important to prepare the appropriate diluted protein standard in the same diluent used for the test protein sample.

FOR STANDARD PROTOCOL (25-2000µg/ml)

2mg/ml BSA Standard (µl)	Diluent (µl)	Final BSA Concentration (µg/ml)
400	0	2000
300	100	1500
200	200	1000
150	250	750
100	300	500
50	350	250
25	375	125
10	390	50
0	400	0 (Blank)



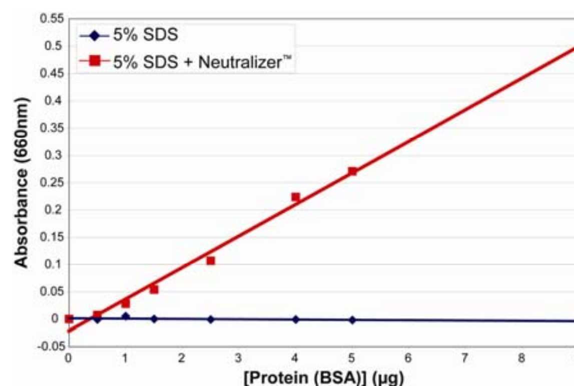


Figure: Presence of Neutralizer™ overcomes 5% SDS interference.

2. SAMPLE PREPARATION

For samples with >0.01% SDS or other interfering ionic detergents

Determine the protein concentration with 10ml RED 660 Protein Assay Reagent supplemented with one vial of Neutralizer™. Simply add and vortex until completely dissolved. This solution is stable for 1 day at room temperature.

For samples in Laemmli loading buffer

Samples directly lyzed in Laemmli buffer, should be diluted 1:10 to 1:20 in Laemmli buffer and the protein concentration determined with 10ml RED 660 Protein Assay Reagent supplemented with one vial of Neutralizer™. Simply add and vortex until completely dissolved. This solution is stable for 1 day at room temperature.

For samples in RIPA Buffer (Cat. # 786-490)

For samples in RIPA buffer, add Triton® X-100 to a final concentration of 0.8%. For example for a 100µl assay sample, combine 92µl RIPA buffer lysed sample with 8µl 10% Triton® X-100. Perform assay as described and multiply the protein concentration by the dilution factor (1.087).

3. STANDARD MICROPLATE OR MICROWELL ASSAY:

We recommend that the assays are performed in duplicate.

1. Transfer 10µl diluted standards, blank and test samples into microwells.
2. Add 200µl RED 660 Protein Assay Reagent into each well and mix well by pipetting up and down.
3. Incubate at room temperature for 5 minutes for optimal results.
4. Vortex samples and then immediately read optical density of the assay tubes at 660nm.
   NOTE: *If a 660nm filter is unavailable, the assay can be read between 645-670nm, however this will result in a decrease in the linear range and also result in a decrease insensitivity.*
5. Subtract the average absorbances at 660nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.
   NOTE: *If a curve-fitting algorithm is used when reading microwell plates on a plate reader, we recommend using a quadratic or best-fit curve for more accurate results. than a purely linear fit.*

4. STANDARD TUBE ASSAY:

We recommend that the assays are performed in duplicate.

1. Transfer 50µl diluted standards, blank and test samples into microwells.
   NOTE: *Smaller sample volumes can be used as long as a ratio of 1:20 Sample to RED 660 Protein Assay Reagent is maintained.*

2. Add 1ml RED 660 Protein Assay Reagent into each well and mix well.
3. Incubate at room temperature for 5 minutes for optimal results.
4. Vortex samples and then immediately read optical density of the assay tubes at 660nm.
   NOTE: *If a 660nm filter is unavailable, the assay can be read between 645-670nm, however this will result in a decrease in the linear range and also result in a decrease insensitivity.*
5. Subtract the average absorbances at 660nm of the blank samples from the average test samples and plot a standard curve for determination of protein concentration of unknown samples.
   NOTE: *If a curve-fitting algorithm is used when reading microwell plates on a plate reader, we recommend using a quadratic or best-fit curve for more accurate results. than a purely linear fit.*

INTERFERENCE TO PROTEIN ASSAY

The following table lists the agents compatible with the RED 660 Protein Assay. The table also shows the acceptable concentration of reagents for standard protocols. In most cases, using a correct blank will eliminate or minimize the error caused by interference. * Indicates acceptable concentration when RED 660 Protein Assay Reagent is supplemented with Neutralizer™.

Table 2: Maximum compatible substances for RED 660 Protein Assay

Compounds		Compounds		Compounds	
Acetone	50%	EDTA	20mM	Octylthioglucopyranoside	10%
Acetonitrile	50%	EGTA	20mM	Octyl-β-glucoside	5%
Ammonium sulfate	125mM	Ethanol	50%	Phenol red	0.5mg/ml
Ascorbic acid	500mM	FOCUS™ Extraction Buffers	Compatible	PIPES, pH6.8	100mM
Bacterial PELB™	Dilute 2-fold	Glutathione (Reduced)	100mM	Sodium acetate, pH4.8	100mM
Borate buffer, pH8.5	50mM	Glycerol	50%	Sodium chloride	1.25M
Brij® 35	5%	Glycine buffer. pH2.8	0.1M	SDS	0.0125%, 5%*
Carbonate-bicarbonate, pH9.4	Dilute 3-fold	Guanidine.HCl	2.5M	Sodium hydroxide	0.125M
CHAPS	5%	HEPES, pH7.5	0.1M	Sucrose	50%
CHAPSO	4%	HCl	125mM	TCEP	40mM
Citrate	12.5mM	Imidazole, pH7.0	200mM	Thiourea	2M
CTAB*	2.5%	Mammalian PELB™	Dilute 2-fold	Tissue PELB™	Dilute 2-fold
Cysteine	350mM	2-mercaptoethanol	1M	Tris.HCl, pH8.0	250mM
Deoxycholate	0.25%	Methanol	50%	Triton® X-100	1%
DMF	50%	MES, pH 6.1	125mM	Triton® X-114	0.5%
DMSO	50%	MOPS, pH7.2	125mM	Tween® 20	10%
DTT	500mM	Nonidet® P-40	5%	Urea	8M

PROTEIN-TO-PROTEIN VARIATION

Protein-dye complex color is primarily the result of binding of the Coomassie dye to the basic and aromatic amino acid residues, especially histidine, arginine and lysine and to a lesser extent tyrosine, tryptophan and phenylalanine; therefore, the RED 660 Protein Assay shows protein-to-protein variations (Table 1). For greater accuracy, the standard plot should be prepared using a protein sample that has a color response similar to the test sample. Ideally, a pure fraction of the test protein.

Table 1: Protein-to-Protein Variation

Protein	Ratio	Protein	Ratio
Aldolase	0.83	Human Transferrin	0.8
Bovine Gamma Globulin	0.51	α-lactalbumin	0.82
Bovine Pancreas Insulin	0.81	Lysozyme	0.79
BSA (Bovine serum albumin)	1.00	Mouse IgG	0.48
Horse Heart Cytochrome C	1.22	Ovalbumin	0.54
Horse Heart Myoglobin	1.18	Rabbit IgG	0.38
Human IgG	0.57	Soybean Trypsin Inhibitor	0.38

TROUBLESHOOTING:

Issue	Suggested Cause	Solution
Lower than expected readings	Wavelength used is incorrect	Measure at 660nm, or between 645-670nm
A precipitate is seen in the assay tubes/ wells	Samples incubated with reagent for more than 5 minutes	Use a 5 minute incubation
		Mix samples by pipetting and read immediately
	DNA and/or RNA are present in the samples	Add Triton® X-100 to a final concentration of 0.8%
Blank is >0.25	Interfering agents present	See Table 2 for suggested concentrations
	Incorrect storage temperature for RED 660 Protein Assay Reagent	Store at room temperature
Assay color is darker than expected	Protein concentration too high	Dilute samples

RELATED PRODUCT

Non-Interfering Protein Assay™ *- A protein assay that overcomes interference of agents commonly present in protein solutions and shows no protein-to-protein variation. This assay has been extensively tested to work in the presence of common laboratory agents, such as reducing agents (2ME, DTT), chelating agents EDTA, detergents, Tris, urea, guanidine hydrochloride, and numerous other agents. The Non-Interfering Protein Assay™ is not affected even if your protein is in extraction buffers containing [4M urea, 1% SDS, 10mM EDTA, and 0.8% 2ME], [1% Sarcosyl, 4M guanidine thiocyanate, 10mM EDTA, and 0.5% 2ME] and other strong extraction buffers.*

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*

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